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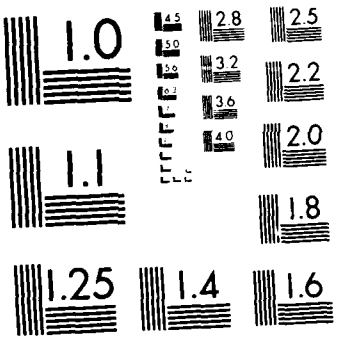
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Electrical field dependence of protein conformation and channel function in lipid membranes of different compositions.

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The key problem tackled in the current research was the behavior of membrane component under the influence of transmembrane or tangential fields. The transmembrane electric field affects the structure and the permeability of the membrane as a whole as well as the conformation of specific membrane components and interacting groups. The effect on the properties of monolayers has been inferred from the measured capacitance and permeability of monolayers transferred to the polarized mercury/water interface. Ionic permeability of unilamellar vesicles upon applying transmembrane electric potentials served for monitoring of the interaction between the membrane components and of its field dependence. The change in conformation of specific components or groups was investigated by measuring circular dichoism and infra red spectra. Electric field below electroporation level (100V/cm) were applied on swollen thylakoid vesicles ~4μm in diameter to induce electrophoretic mobility of their membrane components including photosystem I. The electrophoretic mobility of the latter has been determined by measuring electrostimulated photoluminescence (EPL) from the accumulated and from the depleted photosystems on the two poles of the field polarized vesicles.

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Progress Report on Contract N00014-87-G-0203
Principal Investigator: Israel R. Miller
Title: Electrical field dependence of protein conformation and channel function in lipid membranes of different compositions.

RESEARCH OBJECTIVES

The objective is to study the effect of electrical fields on the conformation of membrane proteins on their lateral distribution and on their function as channels or as receptors. The research has been conducted in three experimental systems, namely lipid monolayers, lipid bilayers (planar or in unilamellar vesicles) and in whole biological membranes.

PROGRESS DURING THE FIRST YEAR STARTING JULY 1987

1. Effect of cholera toxin and of tetanotoxin interacting with their ganglioside receptors on the structure and permeability of lipid monolayers and bilayers.

The toxins were injected underneath lipid monolayers containing different gangliosides at concentrations between 0.5% to 5%. Alternatively the lipid mixtures were spread on very dilute solutions of the toxins. The effect of the toxin on the monolayers was assayed by measuring the change in the monolayer capacitance or conductance after their transfer to the mercury water interface. By these criteria the specific interactions of cholera toxin with lipid monolayers containing GM1 and of tetanotoxin with lipid monolayers containing GT1 has been confirmed. Specific interactions caused large increases in the capacitance and in the conductance of lipid monolayers at nanomolar concentrations of the respective toxins. Only negligible effects on these properties could be observed where the monolayers contained gangliosides which were not specific to the respective toxins. The perturbations caused by the ganglioside/toxin interaction in one monolayer of a bilayer do not translocate spontaneously into the other monolayer. This has been shown by measuring the ionic permeability of unilamellar vesicles made of PC + cholesterol containing 3% GM1 upon interaction with choleratoxin added to the outer aqueous phase. No appreciable increase in permeability was observed upon addition of cholera toxin. The permeabilities were considerably lower than those corresponding to the measured conductances of GM1 containing glycerol monoleate planar bilayers interacting with cholera toxin (Tosteson & Tosteson 1987, Nature 275, 142). Partial translocation of the perturbation caused by cholera toxin can be induced by the transmembrane potential positive on the side of the interacting monolayer.

The general objective of this part of the research program is to demonstrate formation of surface complexes with proteins by specific polar interactions, and to study the subsequent incorporation of the complex into the lipid layer to perturb its uniformity and eventually to form channels.

2. Effect of transmembrane potential on the conformation of membrane components. I. Effect on circular dichoism (CD) of bacteriorhodopsin.
Bacteriohodopsin is one of the most investigated membrane proteins but there is no information available on conformational changes induced by imposed electric fields. We measured the effect of membrane potential on the CD of bacteriorhodopsin embedded in unilamellar vesicles. The transmembrane potential was obtained by adding valinomycin when K^+ gradient was maintained across the membrane. CD spectra measured in absence of K^+ gradient and presence of valinomycin and in the presence of K^+ gradients without valinomycin were taken as reference spectra. The results showed that transmembrane electrical fields, irrespective of their direction, lower the degree of α helicity of bacteriorhodopsin. There are probably also changes in β structures and β turns but the calculations based on basic spectra derived from globular proteins give in this respect only ambiguous results. The mechanism of the modification of the protein conformation by the electrical field is not clear, neither is the effect of the composition of the surrounding lipid on it. To corroborate these results and further to elucidate the field effect we started an investigation of the changes in vibrational spectra of different specific groups in the lipids and in the proteins under the influence of electric fields.

3. Determination of lateral mobility of charged membrane components in vesicle or cell suspensions.

Upon application of a direct electric field pulse on a suspension of vesicles containing charged surface component, electrophoretic mobility is induced. Vesicles move with respect to the solution and each charged component on the vesicular surface moves with respect to its surface environment. Electrophoretic movement of charged membrane components has been discussed previously (M.M. Poo, J.W. Lam, N. Ovida, A.W. Chao (1979) Biophys. J 26, 1-22; S. McLaughin, M.M. Poo (1981) Biophys. J. 34, 85-93).

A uniform electric field E. applied to a spherical cell in a conducting fluid is distorted by the cell and the tangential field at the cell surface E_0 producing the electrophoretic face is

$$E\theta = fE \sin\theta \qquad (1)$$

where f is a nummerical factor representing the field distortion (1.5 for a non-conducting sphere) and θ the polar angle. The flux of the charged membrane component across a unit length of a longitudinal circle at angle θ is

$$\frac{dN}{dt} = = mE_\theta \Gamma_\theta = \frac{D\, d\Gamma_\theta}{r\, d\theta} \qquad (2)$$

where Γ_θ = is the surface concentration at angle θ, m and D are the electrophoretic mobility and the diffusion coefficient of the moving component and r is the radius of the cell. At equilibrium dN/dt = 0, and the ratio of the equilibrium concentrations at the two poles gives the simple expression:

$$\left(\frac{\Gamma\pi}{\Gamma_{o\ eq}}\right) = \exp\left(\frac{2E\pi r}{D}\right) \qquad (3)$$

For studying electrophoretic mobility on spherical cell membrane surfaces we used photosystem I on swollen thylakoid vesicles employing their electro-stimulated photoluminesecene (EPL) as a probe. Exposure of preelluminated thylakoid vesicles to high external fields enhances recombination on the pole at which it counteracts charge separation (D.L. Farkas, S. Malkin, R. Korenstein (1984) Biochim. Biophys. Acta 767, 507-514).

We applied trains of msec long pulses 40-80V/cm 200 pulses/second for moving the photosystems on the vesicular surface until they were depleted from one pole and accumulated on the other one. After prepolarization, the cell suspension was illuminated for 0.1 seconds and then a stimulating 100μsec long pulse of 900V/cm was applied and the EPL measured. The stimulating field enhances charge recombination when it is negative on the outer side of the membrane. Hence, when the direction of the stimulating field is in the direction of the polarizing prepulses it results in lower stimulated luminescence and when it is in the opposite direction it results in higher luminescence than without prepolarization. In Fig. 1 stimulated luminescence in the presence of prepulses 40, 60 and 80 V/cm (60U/cm) of different total duration divided by the stimulated luminescence without any prepulse (I_v/I_o) is given. Values higher than 1 were obtained when the direction of the prepulse was opposite to the direction of the stimulating pulse. Equilibrium for 60V/cm is reached at times longer than 3-4 seconds corresponding to ~0.7 sec polarization time. The concentration ratio of the photosystme on the two poles (equal to the ratio of the luminescence for the opposite prepolarization) becomes to about 5 (2Dmr/D) according to Eqn. 3 becomes then about 1.6.

In Fig. 2 the fraction of EPL decay on the enriched pole or the EPL recovery on the depleted pole is given as a function of time. After a delay of about 90 sec a decay and recovery time constant of about 30 sec is obtained irrespective of the prepolarization potential or if dextrane was added to increase the aqueous viscosity. This corresponds to a diffusion coefficient of about $2 \cdot 10^{-8}$ $cm^2 sec^{-1}$. Emr/D for E=60V/cm being equal to 0.8m comes out to be about $1.4 \cdot 10^{-6}$ $cm^2 V^{-1} sec^{-1}$. This value of m corresponds to a polarization saturation time of about 4 sec for vesicle $2.5 \cdot 10^{-4}$cm in radius, which is about five times higher than the measured ones.

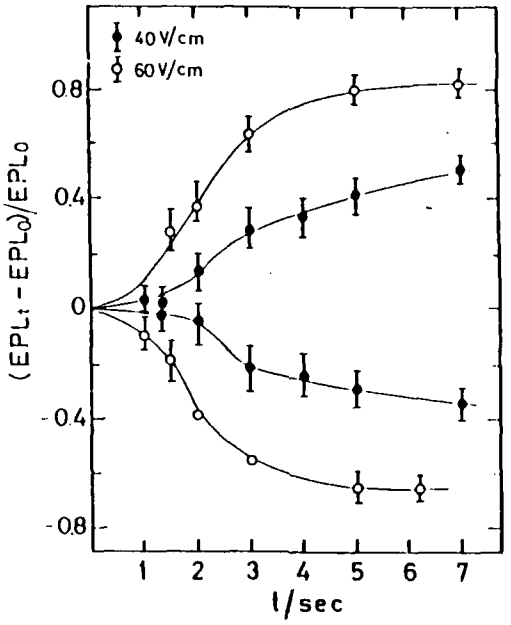

Fig. 1. Field polarization kinetics or increase of EPL on one pole and decrease on the other one as a funciton of time at 40V/cm and 60V/cm.

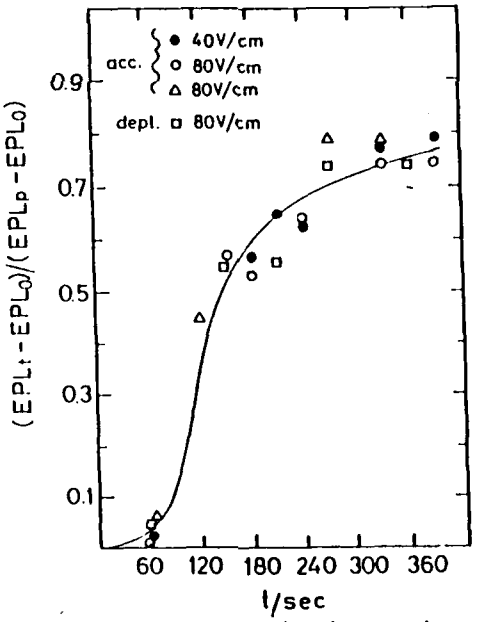

Fig. 2. Relaxation of field polarization. ♦, ϕ and ♦ EPL decay after polarization of 40V/cm ♦, at 80V/cm ϕ at 80V/cm in the presence of dextrane ♦, PL recovery on the depleted pole after polarization at 80V/cm ϕ.

FUTURE PROSPECTIVES AND GOALS

The future research on this project will be conducted along the same lines as proceeded during the first year with the following modifications. The effect of interacting proteins on the structure and the permeability of monolayer and bilayer membranes will be investigated with low molecular channel forming peptides like mellithin, alamethicin etc. Preliminary experiments have been started with mellithin and they look very instructive. We started also FTIR investigation of conformational changes of lipids and membrane proteins under the influence of transmembrane potentials. These investigations proceeded till now at a very slow pace because of lack of dedicated facilities. This will be remedied next month when we shall obtain our new Perkin Elma 1640 FTIR Spectrophotometer, purchased with funds diverted from this grant and with departmental assistance. The investigation of lateral electrophoretic mobility will be attempted on bilayer vesicles between 30 and up to 1000nm in diameter using lipids or proteins tagged with fluorescence probes, e.g. carboxyfluorescencein. In this case fluorescence quenching will be measured as the probes are concentrated on one pole under the influence of the field and then its recovery when the probes are randomized after relaxing the field.

Publications

So far two publications related to this project are in press, one manuscript was submitted and one is in advanced state of preparation.

1. Effect of Membrane Potential on the Conformation of Bacteriorhodopsin reconstituted in Lipid Vesicles. V. Brumfeld & I.R. Miller. Biophysical J. in press.
2. Effect of Interactions in the Head Group on Monolayer Structure and Permeability. I.R. Miller & E. Yavin. Bioelectrochemistry & Bioenergetics in press.
3. Cholera Toxin Complexes with GM1 in Lipid Monolayers and Bilayers: Effect on Structure and Permeability. I.R. Miller, H. Vinkler & E. Yavin. J. Membr. Biology submitted.
4. Electrophoretic Mobility of Proteins in the Plane of Swollen Thykaloid Vesicles. V. Brumfeld, R. Korenstein & I.R. Miller in preparation.

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